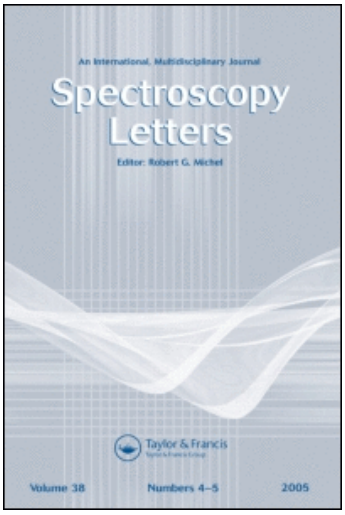


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# PDMS Microfluidic Device with an Integrated Optical Sensor for Determination of Zinc (II) in Pharmaceuticals: Toward the Production of Miniaturized Green Chemistry Analytical Systems



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**ABSTRACT** A simple miniaturized system was fabricated in general laboratory from polydimethylsiloxane (PDMS) according to a prototype casting and molding process permitting the construction of three-dimensional (3D) microchannel (100 μm i.d.) with T-type network, together with an integrated optical sensor for on chip detection. The fabricated device has been successfully applied as a green chemistry-compatible miniaturized analytical system for the determination of zinc (II) in pharmaceutical samples taking the advantage of the colorimetric reaction of zinc (II) and xylenol orange in acetate buffer at pH 5.5. The microfluidic device is a convenient way to quantify zinc (II) over a wide dynamic range, 0.2–2.5 μg $mL^{-1}$, with good linearity ($R^2$ 0.9940).

**KEYWORDS** Green spectrometry, miniaturized system, PDMS, zinc

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## INTRODUCTION

Nowadays, countless analytical methods are well established for environmental monitoring; however, many of these analytical methodologies could contribute to further environmental problems, due to their hazardous reagent consumption and waste generation. Therefore, a focus of Green Analytical Chemistry (GAC) is directed toward the design and development of new analytical procedures that would generate less hazardous substances.[1,2] This goal can be achieved with development of analytical procedures based on the lab on a chip concept such as micro total analysis system (μTAS). Since the concept of μTAS was introduced in 1990 by Manz et al.,[3] a large number of analytical micro-fabricated devices have been developed for environmental monitoring,[4,5] diagnosis,[6] pharmaceuticals,[7] and for detecting terrorist weapons.[8] Microfluidic systems coupled with optical sensor have been applied for

determination of copper (II)[9,10] and iron (III)[11] in water samples; however, these microfluidic systems consisted of sandwich plates fabricated from glass–polydimethylsiloxane (PDMS)[10] or polymethylmethacrylate (PMMA)–PDMS.[9,11]

The production of microfluidic devices usually involves a sequence of many time-consuming microfabrication processes such as photolithography and wet etching, powder blasting, or laser technology.[6,7,12,13] Such processes need high skill and huge investment in infrastructure that might be unaffordable by academic laboratories. Additionally, true $\mu$TAS requires the integration of special instruments such as downscaled pumps[14,15] and on chip detectors.[16,17]

Although there are several manufacturers offering microchip and miniaturized instruments, the cost of these products is still high, especially for low-budget projects. Therefore, we are interested in developing low cost miniaturized systems that can be available in most research laboratories. In this work, a novel low cost casting/molding process enabling the construction of microfluidic devices with 3D cylindrical geometry microchannels from polydimethylsiloxane (PDMS), in general laboratory with no need for specialized instruments, has been demonstrated. The fabricated microfluidic device has been applied as a Green Chemistry-compatible miniaturized analytical system for the determination of zinc (II) in pharmaceutical preparation making use of xylenol orange as the colorimetric discriminatory reagent for zinc (II).



# MATERIALS AND METHODS

## Instrumentation

A peristaltic pump (Ismatec Sa, Switzerland) furnished with a 0.3 mm i.d. PVC pump tubing (Elkay, Hampshire, England) was utilized to deliver reagents and samples to the microfluidic device. Fiber Optic Spectrometer (Ocean Optics. Inc. S2000 with fiber silica cone 0.22 NA and program control OOI Base 32, Mariar Lane, USA) was employed in spectroscopic monitoring.

## Chemicals

Sylgard 184 (Dow Corning, USA) that consists of silicone elastomer and silicone curing agent, was used to prepare PDMS by mixing these two chemicals in a 10:1 ratio.

Zinc (II) stock solution 1000 $\mu$g mL$^{-1}$ was prepared by weighing 0.2200 g of zinc sulphate heptathydrate ($ZnSO_4.7H_2O$) analytical grade (BDH, Poole, England) in RO water and diluting to 50.0 mL. The concentration of zinc (II) stock solution was checked using AAS. The final standards were prepared in acetate buffer pH 5.5.

Xylenol orange stock solution $1.0 \times 10^{-3}$ mol L$^{-1}$, was prepared by dissolving xylenol orange sodium (Lancaster, Morecambe, England) 0.0694 g and diluting to 100.0 mL with RO water, kept in an amber glass bottle in refrigerator. The desired concentration was diluted in RO water.

Acetate buffer 0.5 mol L$^{-1}$ of pH 5.5 was prepared by mixing 500 mL of 0.5 mol L$^{-1}$ of acetic acid (Fisher, Loughborough UK) and 0.5 mol L$^{-1}$ of sodium hydroxide (BDH, Poole England), adjusting to exact pH by 1 mol L$^{-1}$ of hydrochloric acid (Fisher, Loughborough UK) and sodium hydroxide.

Deionized water was prepared by reverse osmosis to a resistivity of $>5$ M$\Omega$ (Elgastat option 4, Elga, High Wycombe, UK).

## Fabrication of PDMS Microfluidic Devices

The microfluidic device was fabricated in house from PDMS according to a prototype casting and molding process allowing the construction of three-dimensional (3D) cylindrical channels. The T-channel microchannel network was constructed from two pieces of nichrome wires (100 $\mu$m diameter). Straight pieces of these wires were held in place via small holes pre-drilled (about 1.5 mm over the bottom) in the side walls of $50 \times 40 \times 30$ mm Perspex mold. These wires were aligned in contact with each other to create a template for the microchannel's manifold. One end of each wire was inserted inside short pieces of PTFE tube (1.6 mm od.). The tubes were extended inside the mold to a distance of 5 mm to cast large channels to be utilized as inlet points for reagents/samples. Near the end of the channel, a third nichrome wire of 200 $\mu$m diameter was fixed vertically at the bottom plate to set up the flow cell template for on chip spectrometric monitoring. The PDMS mixture was prepared by mixing silicone elastomer and curing agent with 10:1 ratio, and poured to fill the mold to the desired level (around 2 cm height) then the mold was placed into

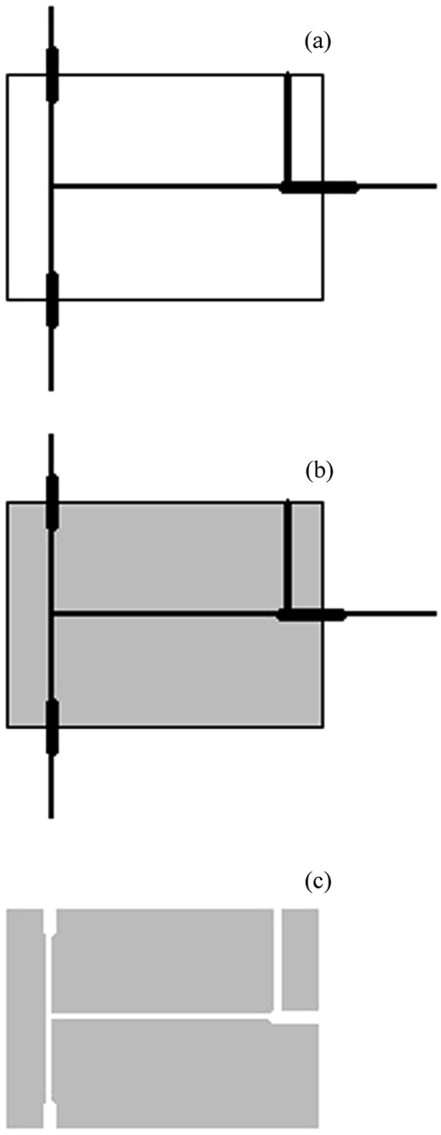


**FIGURE 1** **The PDMS microfabrication process; (a) nichrome wires were fixed in the perspex box, (b) PDMS mixture was poured in the box and then placed in the oven 60°C for 1 hour, (c) Perspex pieces and wires were gently removed.**

the oven to dry at 60°C for 1 h. The mold was then removed from the oven and carefully dissembled to remove the PDMS device and all wires were pulled off leaving microchannels. Afterward the end of the T-channel was blocked with 2 mm diameter iron rod. The fabrication stages of PDMS device are schematically represented in Fig. 1.

## Microfluidic Device with Optical Sensor

The microfluidic system was connected with a fiber optic probe and blue LED as the light source, as demonstrated in the diagram in Fig. 2. The

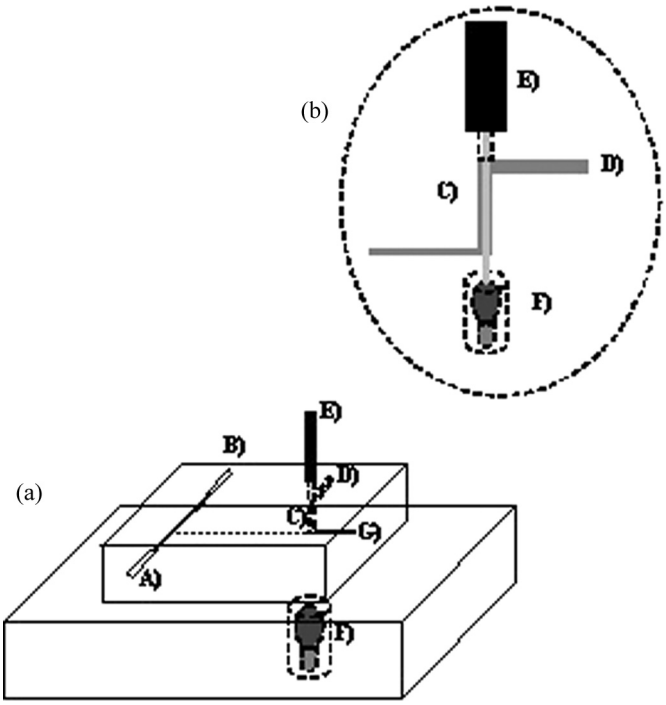


**FIGURE 2** **(a) The microfluidic system incorporated fiber optic (E) and LED (F) as a blue light source fixed at the bottom PMMA plate. The upper plate was PDMS with the micro-flow system that consisted of sample channel A), reagent channel B), flow cell (200 μm × 10 mm) in channel C), waste channel D) and end of T-channel (G) blocked by a piece of steel (2 mm diameter). (b) The enlarged diagram of flow cell and monitoring system.**

reaction of zinc (II) and xylenol orange (XO) in buffer pH 5.5 was used to monitor the color change of complex with variation of zinc (II) concentration. Channels A and B (each 1.6 mm diameter inserted PTFE tube i.d. 0.5 mm and connected to pump tubing PVC, i.d. 0.3 mm) were used for XO and zinc (II) solution, respectively. Zinc (II) and XO were delivered by peristaltic pump with a flow rate of 16 μL $min^{-1}$, mixed in the T-channel on a chip, and then passed through the flow cell (1 cm pathlength) in channel C, where the absorbance was monitored at 570 nm once a stable signal was achieved, and the absorbance was read every 5 s.

## Sample Preparation

Twenty zinc sulphate tablets (a single dosage form that is commonly produced and distributed in Thailand), consisting of zinc sulphate equivalent to 25 mg of zinc per tablet, were weighed and ground. The powder was weighed to 0.1500 g, equivalent to about 17 mg of zinc (II) in a 50-mL volumetric flask and dissolved in 40 mL of water, sonicated for 10 min,

and diluted to 50 mL with water. Then the sample solution was centrifuged at 3000 rpm for 5 min and 30 μL of sample supernatant was pipetted and diluted to 10.0 mL with acetate buffer (about 1 $\mu g\,mL^{-1}$ of zinc (II)). The five different batches of zinc sulphate tablets were selected for zinc (II) determinations.

## RESULTS AND DISCUSSION

This simple fabrication procedure developed in this work was proved to be appropriate for the production of microfluidic devices with basic cylindrical microchannels architecture with three dimensions using low cost materials. The most obvious advantages are that the time consuming and troublesome surface micromachining usually used to produce the master template containing channel architectures, and the technical difficulties associated with bonding stage are eliminated. In this procedure, the problems associated with alignment and bonding were avoided, and the fabricated device exhibited higher mechanical strength and was thus more appropriate for high-pressure applications. The metal wires also provide cylindrical channels, which facilitate the fluidic connection to be accomplished by making use of fused silica capillaries. However, in this work PTFE tubes were employed as templates to cast the connection points. Since PDMS is an elastic material, tubes, capillaries, or a fiber optic probe can be inserted into channels with 50% smaller diameter, resulting in minimal dead volumes. This offers potential advantages when the microfluidic device is interfaced with flowing systems, facilitating sample/reagent introduction. The microfabrication process developed in this work comes in agreement with green chemistry themes because there are no toxic wastes generated from the procedure. It is much safer when compared with photolithography and wet etching. In this article, the fabrication process is applied to the design of simple manifolds such as T-channel networks; however, it could be adapted to allow for the fabrication of more complex structure manifolds.

### Study Signal Monitoring

In this experiment, zinc (II) was determined using the reaction of zinc (II) with xylenol orange (XO) as reported by Benamor et al.[18] and Staden and Tlowana.[19] The red complex of Zinc-XO was scanned in the range 350–700 nm and the maximum wavelength was 570 nm. The effect of surfactants such as sodiumdodecyl sulphate, cetyltrimethylammonium bromide, and triton X-100 as sensitivity enhancer were studied, but no significant increase in the absorbance signal was observed using these media. Therefore, in the on-line determination, only XO and standard solutions of zinc (II) were propelled using the peristaltic pump and mixed in the microchannels at the T-junction. The colored complex that formed in the straight channel was monitored at 570 nm and the blue LED was selected as the light source.

The time scanning of 0.4, 1.2, and 2.0 $\mu g\,mL^{-1}$ standard zinc (II) in pH 5.5 acetate buffer reacted with $2.0 \times 10^{-4}\,mol\,L^{-1}$ XO is shown in Fig. 3. The signal increased steadily for the duration of 100 s, then it started to be stable for 30 s. When the flow was stopped, the signal raised progressively before reaching a plateau yet again. In addition, the blank also showed an absorbance signal when the flow was stopped. Therefore, the blank signal needed to be subtracted from the signals of analytes and this subtracted signal was then used to calculate for linearity for the three concentrations.

When the steady signal at 100–130 s before stopping pump is used, a linear calibration curve with equation $Abs = 0.0819\,[Zn^{2+}] + 0.0511$ with $R^2$ 1.000 was obtained. When the stable signal (at 150–180 s) after stopping flow is used, the equation of the linear graph was $Abs = 0.1698\,[Zn^{2+}] + 0.0500$ with $R^2$ 0.9992. Clearly, the sensitivity during stopped flow (150–180 s) was twice higher than for dynamic flow (i.e., 100–130 s). Stopping the pump not only

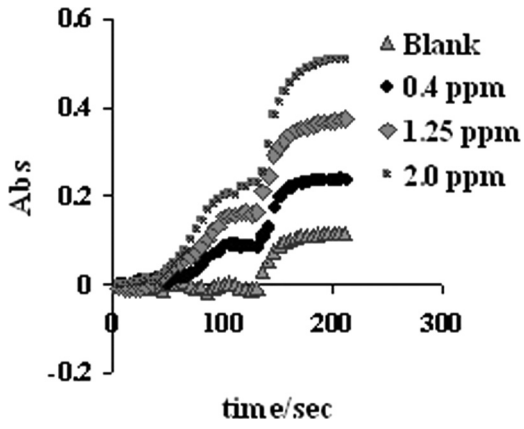


**FIGURE 3** Time scanning for reaction of zinc (II) at concentration 0.4, 1.2 and 2.0 $\mu g\,mL^{-1}$; and XO $2.0 \times 10^{-4}$ mol $L^{-1}$ with flow rate of each line 16 $\mu L\,min^{-1}$ and stopped pump at 130 second.

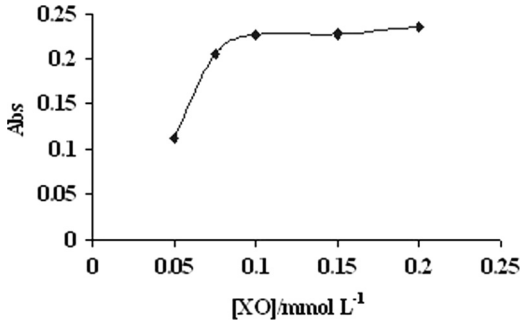


**FIGURE 4** Effect of concentration of xylenol orange: [Zinc(II)] = 1.2 μg mL$^{-1}$.

increases the sensitivity but also reduces the uncertainty of signal from pulse of peristaltic pump. Therefore, for further study, stop flow mode at the optimum timing has been used.

## Effect of XO

The effect of concentration of XO was studied in the range 0.05–0.20 mmol L$^{-1}$ using 1.2 μg mL$^{-1}$ of zinc (II) standard solution in acetate buffer 5.5. Fig. 4 demonstrates the effect of XO and the optimal concentration of XO was determined to be $1.0 \times 10^{-4}$ mol L$^{-1}$.

## Method Validation

Linearity was studied in the range of 0.2–4.0 μg mL$^{-1}$ of zinc (II). The calibration graph was linear over the range $0.2 - 2.5$ μg mL$^{-1}$ and the linear regression was calculated as shown in Table 1. The error in the slope and intercept of the regression line in terms of standard deviation of slope and intercept[20] are also presented in the table. The limit of detection calculated by Miller's method,[20] was 0.18 μg mL$^{-1}$.

**TABLE 1** Linearity Study and Detection Limits of Zinc (II) in a Miniaturized System

| Analytical parameter | Results |
|---|---|
| Linearity range (μg mL$^{-1}$) | 0.20–2.5 |
| Slope $\pm$ $S_b$[a] | $0.1829 \pm 0.0050$ |
| Intercept $\pm$ $S_a$[a] | $0.0289 \pm 0.0069$ |
| $R^2$ | 0.9940 |
| %RSD (N = 10) | 1.3 |
| LOD[b] (μg mL$^{-1}$) | 0.18 |

[a]$S_a$ and $S_b$ is standard deviation of intercept and slope, respectively.
[b]LOD: Limit of detection by Miller's method.

**TABLE 2** Accuracy by Standard Addition of Zinc (II) in Zinc Sulphate Tablets

| No. | Amount add/μg mL$^{-1}$ | Amount found/ μg mL$^{-1}$ | recovery (%) |
|---|---|---|---|
| 1 | 0.25 | 0.25 | 100.0 |
| 2 | 0.50 | 0.51 | 101.2 |
| 3 | 0.75 | 0.74 | 98.1 |
| 4 | 1.00 | 1.03 | 102.9 |
| 5 | 1.25 | 1.23 | 98.5 |

Precision of the system was studied by applying a 1.00 μg mL$^{-1}$ solution of zinc (II) for ten measurements with % RSD 1.3. Accuracy by standard addition method was demonstrated by adding 5 different concentrations (0.2–1.25 μg mL$^{-1}$) in sample solution, and percent recoveries were demonstrated in Table 2.

## Application

Zinc (II) in zinc sulphate tablets (a single dosage of supplements as produced and distributed in Thailand), with five different batches, were determined by the developed miniaturized system. The results were compared with atomic absorption spectrometry (AAS).[21] As can be seen in Table 3, there is no significant difference between the results obtained using the two methods at 95% confidence. Therefore, it is possible to further develop this microfluidic device as an adequate portable miniaturized analytical system for quantity control of raw material of zinc sulphate and zinc sulphate in the production of supplements (in-process control). However, for other applications such as monitoring of zinc (II) in

**TABLE 3** Determination of Zinc (II) in Zinc Sulphate Tablets (Five Different Batches)

| | Quantity of zinc (II)/mg per tablet | |
|---|---|---|
| Number | The proposed method | AAS |
| 1 | 23.4 | 26.0 |
| 2 | 26.5 | 23.8 |
| 3 | 21.8 | 24.0 |
| 4 | 23.1 | 25.9 |
| 5 | 26.4 | 26.9 |
| t-critical[a] | 2.776 | |
| t-experimental | 1.05 | |

[a]95% confidence at degree of freedom = 4.

multi-minerals supplements and pharmaceuticals, sample pretreatment or constituents separation are essential prior to the analysis.

## CONCLUSION

The microfabrication procedure developed in this work is appropriate for the production of microfluidic devices with cylindrical microchannels architecture with 3D using low-cost materials. The outstanding advantages are the elimination of the time consuming and troublesome surface micromachining usually required to produce the master template containing channel architectures, and also the elimination of the technical difficulties associated with the bonding stages. This design of a single PDMS microfluidic device was simple and effectively applied with on chip integrated spectrometric detection for the determination of zinc in zinc sulphate tablets (supplements). With the advantage in small reagent consumption (in microliter), the method fulfills the concept of Green Analytical Chemistry. It is possible to further develop this into a portable and automatic instrument or for in-process control of the pharmaceutical industry.



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## REFERENCES

1. Rocha, F. R. P.; Nóbrega, J. A.; Filho, O. F. Flow analysis strategies to greener analytical chemistry. An overview. *Green Chem.* **2001**, *3*, 216–220.
2. Keith, L. H.; Gron, L. U.; Young, J. L. Green analytical methodologies. *Chemi Rev.* **2007**, *107*(6), 2695–2708.
3. Manz, A.; Graber, N.; Widmer, H. M. Miniaturized total chemical analysis system: A novel concept for chemical sensing. *Sensor Actuators B* **1990**, *1*, 244–248.
4. Crevillén, A. G.; Hervás, M.; López, M. A.; González, M. C.; Escarpa, A. Real sample analysis on microfluidic devices. *Talanta* **2007**, *74*, 342–357.
5. Miró, M.; Hansen, E. H. Miniaturization of environmental chemical assays in flowing systems: The lab-on-a-valve approach vis-à-vis lab-on-a-chip microfluidic devices. *Analytica Chimica Acta* **2007**, *600*, 46–57.
6. Ong, S. E.; Zhang, S.; Du, H.; Fu, Y. Fundamental principles and applications of microfluidic systems. *Frontiers in Bioscience* **2008**, *13*, 2757–2773.
7. Huikko, K.; Kostiainen, R.; Kotiaho, T. Introduction to micro-analytical systems: Bioanalytical and pharmaceutical applications. *European J. Pharmaceutical Sci.* **2003**, *20*, 149–171.
8. Wang, J. Microchip devices for detecting terrorist weapons. *Analytica Chimica Acta* **2004**, *507*, 3–10.
9. Leelasattarathkul, T.; Liawruangrath, S.; Rayanakorn, M.; Liawruangrath, B.; Oungpipat, W.; Youngvises, N. Greener analytical method for the determination of copper(II) in wastewater by micro flow system with optical sensor. *Talanta* **2007**, *72*, 126–131.
10. Kruanetr, S.; Tengjaroenkul, U.; Liawruangrath, B.; Liawruangrath, S. A cleaner and simple spectrophotometric micro-fluidic procedure for copper determination using nitroso-R salt as chromogenic agent. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* **2008**, *70*(5), 1134–1140.
11. Kruanetr, S.; Liawruangrath, S.; Youngvises, N. A simple and green analytical method for determination of iron based on micro flow analysis. *Talanta* **2007**, *73*(1), 46–53.
12. Dittrich, P. S.; Tachikawa, K., Manz, A. Micro total analysis systems. Latest advancements and trends. *Analytical Chem.* **2006**, *78*(12), 3887–908.
13. McCreedy, T. Micro total analytical systems. *Encyclopedia of Analytical Science* **2005**, 10–15.
14. Cooney, C. G.; Towe, B. C. A thermopneumatic dispensing micropump. *Sensors and Actuators A: Physical* **2004**, *116*(3), 519–524.
15. Chen, L.; Wang, H.; Ma, J.; Wang, C.; Guan, Y. Fabrication and characterization of a multi-stage electroosmotic pump for liquid delivery. *Sensors and Actuators B* **2005**, *104*(1), 117–123.
16. Sarov, Y.; Sarova, V.; Ivanov, Tzv.; Ivanova, K.; Capek, I.; Rangelow, I. W. Integrated IR laser system for micro-fluidic detection and analysis. *Chem. Eng. J.* **2008**, *135*, S284–S287.
17. Kang, H.-W.; Lee, I. H.; Cho, D.-W. Development of a micro-bellows actuator using micro-stereolithography technology. *Microelectronic Eng.* **2006**, *83*(4–9), 1201–1204.
18. Benamor, M.; Belhamel, K.; Draa, M. T. Use of xylenol orange and cetylpyridinium chloride in rapid spectrophotometric determination of zinc in pharmaceutical products. *J. Pharmaceutical and Biomedical Analysis* **2000**, *23*(6), 1033–1038.
19. Staden, J. F.; Tlowana, S. I. On-line separation, simultaneous dilution and spectrophotometric determination of zinc in fertilisers with a sequential injection system and xylenol orange as complexing agent. *Talanta* **2002**, *58*(6), 1115–1122.
20. Miller, J. C.; Miller, J. N. *Statistics for Analytical Chemistry,* 3rd edition; Ellis Horwood PTR, Prentice Hall: New York, 1993.
21. The United States Pharmacopoeia XXVII (USP27)–The National Formulary XXII (NF22); The United States Pharmacopeial Convention: Rockville, MD, 2004; pp. 2494.